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USPT,PGPB,JPAB,EPAB,DWPI	cellulose adj binding adj domain	236	L2
USPT,PGPB,JPAB,EPAB,DWPI	hybrid protein	2554	L1

L4 ANSWER 6 OF 9 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.DUPLICATE
ACCESSION NUMBER: 1997:27065127 BIOTECHNO
TITLE: Comparison of the adsorption properties of a single-chain antibody fragment fused to a fungal or bacterial cellulose-binding domain
AUTHOR: Reinikainen T.; Takkinen K.; Teeri T.T.
CORPORATE SOURCE: Dr. T.T. Teeri, VTT Biotechnology and Food Research, PO Box 1500, FIN-02044 VTT, Finland.
SOURCE: Enzyme and Microbial Technology, (1997), 20/2 (143-149), 44 reference(s)
CODEN: EMTED2 ISSN: 0141-0229
PUBLISHER ITEM IDENT.: S0141022996001093
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Trichoderma **reesei** cellobiohydrolase I (CBHI) and Cellulomonas fimi cellulase-xylanase (Cex) both have distinct C-terminal cellulose-binding domains which belong to different CBD sequence families. Two fusion proteins comprising a single-chain antibody fragment (OxscFv) against 2-phenyloxazolone fused to the two CBDs (CBD(CBHI) or CBD(Cex) were constructed. The binding properties of the fusion proteins were studied on different cellulosic substrates. It was shown that the CBD(Cex) binds the fusion protein to cellulose more effectively than the CBD(CBHI); however, once immobilized, both fusion proteins could be eluted from cellulose only with denaturing agents or very low or high pH.
Both fusion proteins retained equally well their ability to bind the hapten recognized by their antibody part.

L4 ANSWER 7 OF 9 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.DUPLICATE
ACCESSION NUMBER: 1996:26292984 BIOTECHNO
TITLE: Characterization of a double cellulose-binding domain.
Synergistic high affinity binding to crystalline cellulose
AUTHOR: Linder M.; Salovuori I.; Ruohonen L.; Teeri T.T.
CORPORATE SOURCE: VTT/Biotechnology and Food Research, Box 1500,FIN-02044 VTT, Finland.
SOURCE: Journal of Biological Chemistry, (1996), 271/35 (21268-21272)
CODEN: JBCHA3 ISSN: 0021-9258
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Most cellulose-degrading enzymes have a two-domain structure that consists of a catalytic and a cellulose-binding domain (CBD) connected by a linker region. The linkage and the interactions of the two domains represent one of the key questions for the understanding of the function of these enzymes. The CBDs of fungal cellulases are small peptides folding into a rigid, disulfide-stabilized structure that has a distinct cellulose binding face. Here we describe properties of a recombinant double CBD, constructed by fusing the CBDs of two Trichoderma **reesei** cellobiohydrolases via a linker peptide similar to the natural cellulase linkers. After expression in Escherichia coli, the protein was purified from the culture medium by reversed phase

chromatography and the individual domains obtained by trypsin digestion. Binding of the double CBD and its single CBD components was investigated on different types of cellulose substrates as well as chitin. Under saturating conditions, nearly 20 .mu.mol/g of the double CBD was bound onto microcrystalline cellulose. The double CBD exhibited much higher affinity on cellulose than either of the single CBDs, indicating an interplay between the two components. A two-step model is proposed to explain the binding behavior of the double CBD. A similar interplay between the domains in the native enzyme is suggested for its binding to cellulase.

L4 ANSWER 8 OF 9 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.DUPLICATE
ACCESSION NUMBER: 1995:25235849 BIOTECHNO
TITLE: Comparison of a fungal (family I) and bacterial (family II) cellulose- binding domain
AUTHOR: Tomme P.; Driver D.P.; Amandoron E.A.; Miller Jr. R.C.; Antony R.; Warren J.; Kilburn D.G.
CORPORATE SOURCE: Dept. of Microbiology/Immunology, University of British Columbia, 300-6174 University Blvd.,Vancouver, BC V6T 1Z3, Canada.
SOURCE: Journal of Bacteriology, (1995), 177/15 (4356-4363) CODEN: JOBAAY ISSN: 0021-9193
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A family II cellulose-binding domain (CBD) of an exoglucanase/xylanase (Cex) from the bacterium Cellulomonas fimi was replaced with the family I CBD of cellobiohydrolase I (CbhI) from the fungus Trichoderma **reesei**. Expression of the hybrid gene in Escherichia coli yielded up to 50 mg of the **hybrid protein**, CexCBD(CbhI), per liter of culture supernatant. The hybrid was purified to homogeneity by affinity chromatography on cellulose. The relative association constants (K(r)) for the binding of Cex, CexCBD(CbhI), the catalytic domain of Cex (p33), and CbhI to bacterial microcrystalline cellulose (BMCC) were 14.9, 7.8, 0.8, and 10.6 liters g.sup.-.sup.1, respectively. Cex and CeXCBD(CbhI) had similar substrate specificities and similar activities on crystalline and amorphous cellulose. Both released predominantly cellobiose and cellotriose from amorphous cellulose. CexCBD(CbhI) was two to three times less active than Cex on BMCC, but significantly more active than Cex on soluble cellulose and on xylan. Unlike Cex, the **hybrid protein** neither bound to .alpha.-chitin nor released small particles from dewaxed cotton fibers.

L7 ANSWER 7 OF 12 BIOTECHNO COPYRIGHT 2002 Elsevier Science B.V.DUPLICATE

ACCESSION NUMBER: 1995:25230420 BIOTECHNO

TITLE: **PEG**-BP-30 monotherapy attenuates the cytokine-mediated inflammatory cascade in baboon Escherichia coli septic shock

AUTHOR: Espat N.J.; Cendan J.C.; Beierle E.A.; Auffenberg T.A.; Rosenberg J.; Russell D.; Kenney J.S.; Fischer E.; Montegut W.; Lowry S.F.; Copeland III E.M.; Moldawer L.L.

CORPORATE SOURCE: Department of Surgery, Univ. of Florida College of Medicine,Gainesville, FL, United States.

SOURCE: Journal of Surgical Research, (1995), 59/1 (153-158) CODEN: JSGRA2 ISSN: 0022-4804

DOCUMENT TYPE: Journal; Conference Article

COUNTRY: United States

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Septic shock following gram-negative infection is a leading cause of mortality in critically ill patients, accounting for nearly 200,000 deaths a year. The exaggerated production of tumor necrosis factor-.alpha. (TNF.alpha.) is known to contribute to hemodynamic collapse and the hematological dyserasia associated with gram-negative sepsis. Although previous studios have shown TNF.alpha. antibodies and TNF immunoadhesins to be effective in experimental gram- negative sepsis, we postulated that administration of a novel construct of two modified soluble p55 receptors **linked** to polyethylene glycol (**PEG**-BP-30) would also attenuate the hemodynamic and hematologic alterations to lethal Escherichia coli septic shock in nonhuman primates.

Nine adult female and male baboons (Papio anubis), weighing 10-17 kg, were anesthetized and invasively monitored. The nine animals were randomized to receive either 0.2 mg/kg body wt **PEG**-BP-30 (n = 3), 5.0 mg/kg body wt **PEG**-BP-30 (n = 3), or placebo (n = 3). One hour after pretreatment, animals were infused with 5-10 x 10.sup.2.sup.0 CFU/kg of live E. coli iv and vital signs were recorded for the next 8 hr. Arterial blood was drawn for baseline parameters and throughout the study to obtain total and differential white blood cell and platelet counts and cytokine levels (TNF.alpha., IL-1.beta., IL-6, IL-8). E. coli bacteremic baboons receiving only placebo demonstrated a significant fall in mean blood pressure and leukopenia. Two of the three animals expired. In contrast, five of the six baboons receiving the **PEG**-BP-30 survived and these animals exhibited markedly attenuated declines in blood pressure and leukocyte numbers. Septic baboons also manifested monophasic plasma TNF.alpha., IL-1.beta., IL-6, and IL-8 responses that were significantly attenuated by **PEG** -BP-30 pretreatment in a dose-dependent manner. We conclude from these data that the administration of **PEG**-BP-30 improves survival and attenuates the TNF.alpha.-